Consultants in Veterinary Pathology, Inc.
P.O. Box 68 Murrysville, PA 15668-0068
Phone: (724) 733-5154 Fax: (724) 733-3032 E-mail: vetpathol@cs.com

Memorandum

To: Ray York, Joanne Fairlie, John Barnett Jr., Joan Dollarhide, Michael Dourson, Jean Harry, and Jerry Hardisty

From: Bob Garman

CC:

Date: 8/13/01

Re: Ammonium perchlorate study.

Message: After reading the most recent draft protocol and the recent discussions *re:* numbers of dose groups, I would like to offer the following comments:

1) Because of variability in brain size at early time points (day 11 and earlier), I do not feel that eight rats/sex/dose group will be a sufficient number for the morphometric component of this study. (Originally, I thought that there were to be 20/sex/group – i.e. one male and one female from each litter. I subsequently found that there would be 10/sex/group. Now, with the extra dose group, it appears that there will be only eight.)

2) Before finding out about the very tight deadline for this study, I had suggested that we dissect and block all of the brains from the intermediate and low dose groups in order to allay any concerns about possible additional shrinkage after longer term fixation. However, removing the brains from the cranial vaults of young animals is time consuming due to the fragility of these brains and may not be possible within the time constraints imposed.

In order to address these concerns, I would offer the following suggestions:

1) Regarding Item 1, I would suggest that brains be collected from one male and one female from each litter in each treatment group (other than for the control group), thus yielding 16 brains/group rather than 8. For the control group, I would collect four times as many brains (*i.e.* 4 males and 4 females/control group litter). We could then process and section the brains from 8 rats/sex at each time point from the control and high dose group but would retain the option of expanding the group size, later, to a

total of 16/sex/dose. If we should decide to expand the group size or to "read down" to the next dose level, we would have an extra matching set of control brains for each such group that had been fixed for a similar period of time.

2) Regarding Item 2, discussions with a number of colleagues and a brief literature review have failed to indicate any evidence of significant additional tissue shrinkage once fixation is completed. Nevertheless, I feel that it would be prudent to save the additional matching control brains to avoid any potential criticism. To do so would require only a minimal amount of additional time input and would be more time efficient and cost-effective than dissecting, processing, and blocking the brains from the rats in each treatment group. By taking this approach, we could concentrate on the histotechnical aspects of this study (the most critical component) and would be able to enhance the turnaround time. NOTE: If the ranges of the morphometric parameters turn out to be similar for control brains processed at later points in time, these data (from the various control and treatment groups) would be combined. Even if analyzed as separate groups of 8 rats/time point, having these extra control groups would contribute to an understanding of degrees of variability present within groups of this size and age. Such an approach in this study would also help to answer the question as to whether all brains need to be processed at the same point in time (not routinely done on standard developmental neurotoxicity studies).

If this approach is acceptable *re*: brain collection, we need to discuss how this would impact on thyroid collection. Once we decide how long the gestational day 21 and day 5 rats are to be fixed (presumable in Bouin's), the heads/brains from these rats would be washed two times in 30 – 50% alcohol and then changed to formalin. It might make the most sense to have all of the extra heads retained at Argus, although they could be shipped to EPL for removal of the thyroids and subsequent removal and archiving of the brains. Ray and Jerry, we should undoubtedly discuss this further. Before I can work up a modified cost estimate for the neuropathology component of this study, I will need to have confirmation *re:* the numbers of brains to be processed/dose group and to know if we are to process all brains to the paraffin stage or merely collect extra control brains in fixative. I will also need to finalize with Jean the numbers of areas to be measured and whether the NIH image program is to be used for this data collection rather than an ocular micrometer.

I would appreciate any input that each of you may have.

Bob